



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Vilmos Kéri et al.

FWCIP of Serial No. 08/659,961

Filed on herewith

For PROCESS FOR THE ISOLATION AND PURIFICATION, etc.

Attorney's Docket 0100-004

Hon. Commissioner of Patents and Trademarks  
Washington DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to assigning a Serial No to the above-identified application,  
please amend it as follows:

In the disclosure

Page 1, before line 1 insert --This is a continuation-in-part of application Ser. No. 08/659,961 filed on June 7, 1996, which is a continuing application of Ser. No. 08/269,150 filed on June 30, 1994, both abandoned.--

Page 3, line 5, after "Collection)" insert --and other bacterial strains, such as *Aspergillus terreus*) accessible under ATCC 20542).--

Page 14, after the end of the page add the following new Examples:

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Example 7.

800 g fermentation liquor cultured by an *Aspergillus terreus* strain (deposition access No. ATCC 20542) containing a total amount of 630 mg of mevinolin both as lactone and as hydroxy acid were diluted to 1,200 g. with water. Then 2.4 g ethylene glycol were added to the mixture, and the pH was maintained at 9 to 9.5 by adding 20% wt. KOH solution under continuous stirring for



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2 hours. The biomass was then filtered off and suspended in 400 cm<sup>3</sup> water. The suspension was adjusted to pH 9-9.5 with 20% wt. KOH solution, filtered again and the filtrates were combined. 1,480 cm<sup>3</sup> of filtered liquor containing 554 mg of active ingredient were obtained. Then 3.5 g of CaCl<sub>2</sub> were added to the liquor and the solution was adjusted to pH 2.1 with 15% wt. sulfuric acid solution under stirring. The separate precipitate was settled for 4 hours and processing was completed as in Example 2, with the difference that the active ingredient was dissolved from the precipitate with 120 cm<sup>3</sup> of isobutylacetate. 370 mg product was obtained. The obtained mevinolin has an active ingredient content of 98% by high pressure liquid chromatography, containing 0.2% dihydromevinolin by HPLC.  $[\alpha]_{25}^{20} = +326^{\circ}\text{C}$  (c=0.5; acetonitrile).

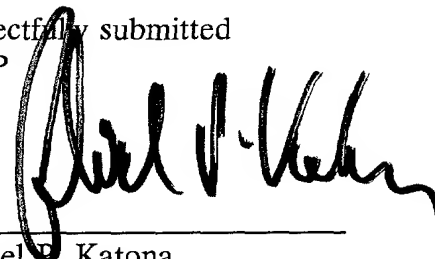
#### Example 8.

800 g of fermentation liquor cultured by an *Aspergillus terreus* strain and containing a total amount of 620 mg of mevinolin, both as a lactone and as hydroxy acid were diluted to 1200 g with water. Then 2.4 ethylene glycol were added to the mixture, and the pH was maintained at 8.5 to 9.0 by adding 20% wt. KOH solution under continuous stirring for 2 hours. The biomass was then filtered off and suspended in 400 cm<sup>3</sup> water containing 0.8 g of ethylene glycol. The suspension was adjusted to pH 8.5-9.0 with 20% wt. KOH solution, filtered again and the filtrates were combined. 1,470 cm<sup>3</sup> of filtered liquor containing 535 mg of the active ingredient were obtained which was adjusted to pH 3.0 with 15% wt. phosphoric acid under stirring. The precipitate was settled over 4 hours. The balance of the process was completed as described in Example 2, resulting in the isolation of 334 mg mevinolin with an active ingredient content of 98.6% by high pressure liquid chromatography, with a dihydromevinolin content of 0.2 % by HPLC.  $[\alpha]_{25}^{20} = +328^{\circ}\text{C}$  (c=0.5; acetonitrile).--

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Respectfully submitted



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100-004

## PROCESS FOR THE ISOLATION AND PURIFICATION OF MEVINOLIN

### Field of the invention

This invention relates to a process for the isolation and purification of mevinolin from fermentation liquor.

Mevinolin, also known as lovastatin, Mevacor, monacolin K, and MK 803 is a known antihypercholesteremic agent, which can be produced by fermentation using either a microorganism such as of the species *Aspergillus terreus* or different microorganism species of the *Monascus* genus, obtained either as an open chain hydroxy acid or as lactone. The compound has the formula

## Background of the invention

The isolation of the active ingredient is suitably carried out either by directly extracting the fermentation liquor with a solvent, or by extracting the filtered liquor and the biomass and subsequently purifying the crude product such as by chromatography.

5 Ethyl acetate, chloroform or benzene can be used for the extraction. The fermentation liquor contains partly the open-chain hydroxy acid of mevinolin, 3,5-dihydroxy-7-[1,2,6,7,8,8a-hexahydro-2,6-dimethyl-8-(2-methylbutyryloxy)-naphthalene-1-yl]-heptanoic acid. This compound is heated in toluene to be lactonized to mevinolin. The purification of the mevinolin containing crude product completely in the lactone form can  
10 be carried out by chromatography and subsequent recrystallization in accordance with the process disclosed in U.S. patent No. 4,319,039.

In addition to extraction an  $XAD_2$  adsorption resin can also be used for the isolation of mevinolin as disclosed in U.S. patents Nos. 4,231,938 and 4,319,039.

15 The main disadvantage of the extraction method is that together with the active ingredient the solvent also dissolves many other contaminants, and thus makes the further purification more complicated and expensive. Purification can be efficiently carried out by multistage column chromatography and subsequent recrystallization.

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Experiments have been carried out to compare the extraction method described in Hungarian patent No. 187,296 to the method according to the present invention for the isolation of mevinolin from fermentation liquor obtained by cultivation of an *Aspergillus obscurus* MV-1 holotype strain (deposit No. NCAIM (P)F 001189 at the Hungarian National Collection). The results, as shown in Example 1, demonstrate that the product obtained from the fermentation liquor by extraction cannot be properly purified by recrystallization. The preparation by this route of a product is not suitable for pharmaceutical purposes, therefore requires further purification by column chromatography.

10

Description of the invention

The object of the present invention is to provide a process for the isolation of mevinolin from a fermentation liquor, which can be carried out more readily and more economically than the known processes, and which enables the preparation of the active ingredient in a quality suitable for pharmaceutical purposes.

15

The present invention is based on the recognition that the active ingredient can be separated with high efficiency directly from the filtered fermentation liquor at a pH between about 4.5 and about 1. The separated crude product does not need to be purified by chromatography, since only a surprisingly small amount of contamination separates together with it. Thus a simple recrystallization is sufficient to obtain a product of suitable quality.

20

According to the process of the present invention the active ingredient is dissolved from the biomass into the fermentation liquor at a pH of from about 7.5 to about 10. The biomass is filtered off, the crude product is separated from the filtered liquor at a pH of from about 4.5 to about 1 and purified by methods known *per se*, suitably by recrystallization.

The separation of the active ingredient has been investigated at different acidic pH values. The pH range of from about 1.8 to about 2.4, especially from about 2 to about 2.2 has been found to be the most suitable. It has also been found that the separation of the active ingredient from the filtered fermentation liquor, and especially the filterability of the precipitate, can be improved by the addition of bivalent or trivalent metal salts, for example earth alkali metal salts such as  $\text{CaCl}_2$ ,  $\text{MgCl}_2$ ,  $\text{MgSO}_4$  or earth metal salts such as  $\text{Al}_2(\text{SO}_4)_3$ , or transition metal salts, such as ferrous, or ferric salts.

The active ingredient content of the filtered fermentation liquor is shown in the table below with the active ingredient having been filtered off at varying pH with or without adding calcium chloride to the filtered liquor. The active ingredient content was determined by high pressure liquid chromatography.

Active ingredient content of the filtered liquor ( $\mu\text{g}/\text{cm}^3$ )

	pH	without adding any salt	in the presence of 0.2 M $\text{CaCl}_2$
5	7	418	60
	6	387	65.8
	5	201	103
	4	58	50
	3	31	22
10	2	14	10
	1.5	10	10
	1	8	8

As the majority of the active ingredient is bound to the biomass, both the efficiency of the dissolution into the fermentation liquor and the amount of the contaminants are of significance.

It has also been recognized that by carrying out the dissolution of the active ingredient into the fermentation liquor at a pH between about 7.5 and about 10, more particularly between about 8 and about 9, both the loss of the active substance and the amount of the contaminations can be kept at a minimum.

It was experimentally found that the dissolution of the active ingredient can be enhanced by adding a small amount of additives to the mixture.  $\text{C}_{1-4}$  aliphatic alcohols,  $\text{C}_{2-5}$  glycols,  $\text{C}_{1-3}$  secondary or tertiary amines,  $\text{C}_{1-5}$  alkyl acetates, dimethylformamide,

polyethylene glycol, or polypropylene glycol have been found to be particularly suitable additives, and ethylene glycol and ethanol are most suitable additives.

The active ingredient content of the filtered fermentation liquor is shown in the following table before the separation of the active ingredient at about pH 9 and after the filtration thereof at about pH 2, both with and without additives.

Additive	Active ingredient in filtered form liquor ( $\mu\text{g}/\text{cm}^3$ )	
	@ pH 9	@ pH 2
1% vol.		
diethylamine	412	9.2
triethylamine	423	10.5
dimethylformamide	460	6.9
methanol	429	7.9
ethanol	455	11.2
isopropanol	467	8.7
ethylene glycol	467	5.1
propylene glycol	450	10.2
polypropylene glycol	369	19.1
isobutyl acetate	258	8.8
polyethylene glycol	431	11.8
control (without additive)	193	8.6

It is clear from the foregoing table that the active ingredient content of the filtered liquor is higher when additives are used than without the use of the additives. Thus the additives promote the dissolution of the active ingredient from the biomass into the fermentation liquor. It can also be seen that the additives do not have any influence on the separation because that can be accomplished with the same efficiency with or without



the use of additives. Their use, is therefore suitable since they amplify the procedure, because a single forming of a suspension from the biomass is sufficient. On the other hand, when no additives are used then the procedure has to be repeated to achieve the same efficiency.

As shown in the next table, the additives perform well, even when employed at a small concentration, such as about 0.1% volume based on the fermentation liquor.

concentration of ethanol % vol.	active ingredient content of the filtrate liquor ( $\mu\text{g}/\text{cm}^3$ )	
	@ pH 9	@ pH 2
0.1	400	8.9
0.5	425	8.5
1.0	455	11.2
5.0	447	11.5
10.0	441	13.0
15.0	434	18.1
20.0	430	26.0

The crude product can be purified by any suitable method, such as by recrystallization. Crystallization is suitably carried out from isobutyl acetate by washing the isobutyl acetate solution of the substance with a weakly basic 2.5% wt. ammonium sulfate solution adjusted to about 8.5 pH, the solvent phase is clarified with carbon, concentrated and the separated product is filtered off.

5 The present process enables elimination of the extraction of both the fermentation liquor and the biomass from the procedure, the active ingredient separated from the filtered fermentation liquor at acidic pH is surprisingly pure, and so it does not require to be purified by chromatography, but simple recrystallization leads to a product of acceptable pharmaceutical purity. Therefore the process is simple and can be economically accomplished with only a slight loss of substance, at yields above 90%.

The invention is further illustrated by the following examples.

#### Example 1

10 A comparative experiment was carried out by using as comparison the extraction method specified in Hungarian patent No. 187,296.

15 800 g of fermentation liquor cultured by an *Aspergillus obscurus* MV-1 holotype strain, deposition access No. NCAIM (National Collection of Agricultural and Industrial Microorganisms) (P)F 001189) containing a total of 670 mg of mevinolin both as lactone and as hydroxy acid were adjusted to pH 4 with 20% wt sulfuric acid solution. The liquor was then extracted with 400 cm<sup>3</sup> of ethyl acetate. The organic phase containing the active ingredient was separated and the aqueous residue was extracted again with further 400 cm<sup>3</sup> of ethyl acetate. The ethyl acetate extracts were combined to yield 760 cm<sup>3</sup>, containing 643 mg of the active ingredient, dried over anhydrous sodium sulfate and concentrated under vacuum. The concentrate was boiled in 100 cm<sup>3</sup> toluene for 2 hours. Then the

undissolved particles were filtered off and washed successively with 50 cm<sup>3</sup> of 5% wt sodium hydrogen carbonate solution and 50 cm<sup>3</sup> of water. The toluene solution was dried over anhydrous sodium sulfate and evaporated under vacuum. The thus obtained 3.5 g oily product contained 630 mg of the active ingredient. The oily product was dissolved by warming in 15 cm<sup>3</sup> of ethanol and allowed to stand at a temperature of 5°C for 24 hours. The product did not separate out in crystalline form. The solvent was then removed and the oily product (3.5 g) was divided into two parts.

1.75 g of product was attempted to be recrystallized from 6 cm<sup>3</sup> of isobutyl acetate as specified in Example 2. The product did not separate out in crystalline form.

The other part of the product was subjected to column chromatography using a 22 cm long column having a 1.6 cm diameter packed with 20 g of a silicagel sold under the trademark Kieselgel 60 having a particle size of from 0.063 to 0.2 mm. The column was eluted with a 40 : 60 mixture of ethyl acetate and methylene chloride at a rate of 20 cm<sup>3</sup>/hour. The 6 to 10 fractions containing the active ingredient were combined, clarified with activated carbon, filtered and evaporated under vacuum to yield 260 mg of yellowish white solid residue, which was recrystallized from ethanol. The separated crystals were filtered through a G-4 sieve, washed with 10 cm<sup>3</sup> of n-hexane and dried under vacuum at room temperature. A first batch of 180 mg of chromatographically pure mevinolin were obtained.

The evaporation residue of the mother liquor obtained during the crystallization was recrystallized again from ethanol to obtain a second batch of 35 mg mevinolin. The quality of the second batch of product was the same as that of the first batch.

### Example 2

800 g of fermentation liquor cultured by the *Aspergillus* strain of Example 1 containing a total amount of 536 mg of mevinolin both as lactone and as hydroxy acid were diluted to 1200 g with water. Then the solution was kept at a pH between 8.5 and 9 with 20 % wt potassium hydroxide solution under continuous stirring for 2 hours. The biomass was then filtered off and suspended twice each in 400 cm<sup>3</sup> of water. The suspension was adjusted to a pH between 8.5 and 9 with 20 % wt K OH solution, filtered again and the filtrates were combined. Thus 1900 cm<sup>3</sup> of filtered liquor containing 530 mg of active ingredient was obtained. The liquor was then adjusted to pH 2.1 with 15 % wt sulfuric acid solution, under stirring. The separated precipitate was settled, filtered, suspended in 100 cm<sup>3</sup> sulfuric acid solution adjusted to pH 2 and filtered again. The active ingredient concentration of the filtrate was 12 µg/cm<sup>3</sup>.

The filtered aqueous precipitate was dissolved in 50 cm<sup>3</sup> isobutyl acetate, the aqueous phase was separated and the solvent phase was concentrated to 2.5 cm<sup>3</sup>. The concentrate was dissolved in 60 cm<sup>3</sup> of isobutyl acetate, washed twice each time with 60 cm<sup>3</sup> of an aqueous ammonium sulfate solution adjusted to pH 8.5 with ammonium hydroxide, clarified with 0.5 g of activated carbon, concentrated to 10 cm<sup>3</sup>, allowed to

crystallize for 24 hours at 5°C, filtered and dried under vacuum .436 mg mevinolin were recovered. The active ingredient content was 98.7% by high pressure liquid chromatography. A further 65 mg of mevinolin were obtained from the combined mother liquors, with a purity of 92.8%. The crude products were combined and recrystallized from ethanol, to obtain 450 mg of product with an active ingredient content of 99.8% by high pressure liquid chromatography.

The end product contained 0.17% dihydromevinolin by gas chromatography  $[\alpha]_{25_D} = +329.8^\circ$  (specific optical rotation) according to the method of 22 United States Pharmacopoeia p.3273 with 0.5 g of the material dissolved in 100 ml a acetonitrile.

### Example 3

800 g of fermentation liquor cultured by the *Aspergillus* strain of Example 1 containing a total amount of 605 mg of mevinolin both as lactone and as hydroxy acid were diluted to 1200 g with water. Then 2.4 g of ethylene glycol were added to the mixture, and kept at pH 8.5 - 9.0 by adding 20% wt KOH solution under continuous stirring for 2 hours. The biomass was then filtered off and suspended in 400 cm<sup>3</sup> water containing 0.8 g of ethylene glycol. The suspension was adjusted to pH 8.5 - 9.0 with 20% wt KOH solution, filtered again and the filtrates were combined. 1470 cm<sup>3</sup> of filtered liquor were obtained containing 600 mg active ingredient, which was adjusted to pH 2.1 with 15% wt phosphoric acid under stirring. The precipitate was settled for 4 hours. The balance of the process was completed as described in Example 2, resulting in the isolation

of 548 mg mevinolin with an active ingredient content of 99.7% by high pressure liquid chromatography with a dihydromevinolin content of 0.15% by gas chromatography.

$[\alpha]_{25_D} = +329^\circ$  (c=0.5; acetonitrile)

#### Example 4

5            800 g of fermentation liquor cultured by the *Aspergillus* strain of Example 1 containing a total amount of 575 mg of mevinolin both as lactone and as hydroxy acid were diluted to 1200 g with water. Then 2.4 g ethylene glycol were added to the mixture, and the pH was maintained at 9 to 9.5 by adding 20% wt KOH solution under continuous stirring for 2 hours. The biomass was then filtered off and suspended in 400 cm<sup>3</sup> water. 10            The suspension was adjusted to pH 9 - 9.5 with 20% wt KOH solution, filtered again and the filtrates were combined. 1480 cm<sup>3</sup> of filtered liquor containing 567 mg of active ingredient were obtained. Then 3.5 g of CaCl<sub>2</sub> were added to the liquor and the solution was adjusted to pH 2.1 with 15% wt sulfuric acid solution under stirring. The separated precipitate was settled for 4 hours and processing was completed as in Example 2, with 15            the difference that the active ingredient was dissolved from the precipitate with 120 cm<sup>3</sup> of isobutyl acetate recovering 527 mg mevinolin with an active ingredient content of 99.2 % by high pressure liquid chromatography, containing 0.25% dihydromevinolin by gas chromatography.

$[\alpha]_{25_D} = +329^\circ$  (c=0.5; acetonitrile)

### Example 5

10,000 g of fermentation liquor cultured by the *Aspergillus* strain of Example 1 containing a total amount of 4180 mg of mevinolin both as lactone and as hydroxy acid were diluted to 15,000 g with water. Then 30 g of ethylene glycol were added to the mixture, and kept at pH 8 - 8.5 by adding 20% wt KOH solution under continuous stirring for 2 hours. The biomass was then filtered off and suspended in 5 dm<sup>3</sup> water containing 10 g ethylene glycol. The suspension was adjusted to pH 8 - 8.5 with 20% wt KOH solution, filtered again and the filtrates were combined. Thus 18,200 cm<sup>3</sup> of filtered liquor containing 4,091 mg of active ingredient were obtained. Then 20 g magnesium sulfate were added to the mixture and it was adjusted to pH 2.1 with 15% wt sulfuric acid solution, under stirring. The separated precipitate was settled, filtered, suspended in 1,200 cm<sup>3</sup> of an aqueous sulfuric acid solution, adjusted to pH 2 and filtered again. The filtered aqueous precipitate was dissolved in 600 cm<sup>3</sup> of isobutyl acetate, the aqueous phase was separated and the solvent phase was concentrated to 30 cm<sup>3</sup>. The concentrate was dissolved in 400 cm<sup>3</sup> of isobutyl acetate, washed twice with 400 cm<sup>3</sup> each of 2.5% wt. ammonium sulfate solution adjusted to pH 8.5 with ammonium hydroxide solution, and clarified with 6 g activated carbon by stirring for half and hour at room temperature. The solution was concentrated to 80 cm<sup>3</sup>, allowed to crystallize for 24 hours at 5°C, filtered and dried under vacuum. The balance of the process was coupled as specified in Example 2, recovering 3432 mg mevinolin with an active ingredient content of 99.1% by high

pressure liquid chromatography, containing 0.19% dihydromevinolin by gas chromatography.

$[\alpha]_{25_D} = +328.9^\circ$  ( $c=0.5$ ; acetonitrile)

#### Example 6

5            100 kg of fermentation liquor cultured by the *Aspergillus* strain of Example 1 containing a total amount of 44.3g of mevinolin both as lactone and as hydroxy acid were diluted to 150 kg with water. Then 300 g ethylene glycol were added to the mixture which was kept at pH 8.5 - 9 by adding 20% wt. KOH solution under continuous stirring for 2 hours. The biomass was then filtered off and suspended in 50 kg water containing 100 g of ethylene glycol. The suspension was adjusted to pH 8.5 - 9 with a 20% wt KOH solution, filtered off again and the filtrates were combined. 183 kg filtered liquor containing 42.9 active ingredient were obtained.

10            Then 200 g of magnesium sulfate were added to the liquor and the solution was adjusted to pH 2.1 with 15% wt sulfuric acid solution under stirring. The separated precipitate was settled, filtered, suspended in 12 dm<sup>3</sup> sulfuric acid solution adjusted to pH 15            2, and filtered again. The filtered aqueous precipitate was dissolved in 6 dm<sup>3</sup> isobutyl acetate, the aqueous phase was separated, and the solvent phase was concentrated to 300 cm<sup>3</sup>. The concentrate was dissolved in 4 dm<sup>3</sup> isobutyl acetate, washed twice with 4 dm<sup>3</sup> each of 2.5% wt. ammonium sulfate solution adjusted to pH 8.5 with ammonium hydroxide solution, and clarified with 60 g activated carbon by stirring for half an hour at room 20



temperature. The solution was concentrated to 0.8 dm<sup>3</sup>, allowed to crystallize for 24 hours at 5°C, filtered and dried under vacuum, and the process was concluded as in Example 2, recovering 37.03 g mevinolin, having an active ingredient content of 99.3% by high pressure liquid chromatography and containing 0.18% dihydromevinolin by gas chromatog-  
5 raphy.

$[\alpha]_{25_D} = +329.5^\circ$  (c=0.5; acetonitrile)

We claim:

1           1. In a process for preparing mevinolin by fermentation of a biomass in a  
2 fermentation liquor, which includes dissolving mevinolin from the biomass into the fermenta-  
3 tion liquor, and separating the biomass from the fermentation liquor to obtain a  
4 separated fermentation liquor, separating the mevinolin from the separated fermentation  
5 liquor, and recovering the end product, **the improvement which comprises** carrying out the  
6 dissolving at a pH between about 7.5 and about 10, and the separating of the mevinolin  
7 is carried out at a pH between about 4.5 and about 1.

1           2. The process of claim 1, wherein the dissolving is carried out at a pH  
2 between about 8 and about 9.

1           3. The process of claim 1, wherein the separating of the mevinolin is carried  
2 out at a pH between about 2.2 and about 2.

1           4. The process of claim 2, wherein the separating of the mevinolin is carried  
2 out at a pH between about 2.2 and about 2.

1           5. The process of claim 1, wherein the dissolving is carried out in the  
2 presence of at least about 0.1 % wt. based on the volume of the fermentation liquor of  
3 at least one additive of a C<sub>1-4</sub> aliphatic alcohol, a C<sub>2-5</sub> glycol, a C<sub>1-3</sub> secondary or tertiary  
4 amine, a C<sub>1-5</sub> alkyl acetate, dimethylformamide, polyethylene glycol, and polypropylene  
5 glycol.

1           6. The process of claim 5, wherein said additive is ethanol, or ethylene  
2 glycol.

1           7. The process of claim 5, wherein the additive is diethylamine, triethylamine,  
2 dimethylformamide, methanol, isopropanol, ethylene glycol, propylene glycol, polypropylene  
3 glycol, isobutyl acetate, and polyethylene glycol.

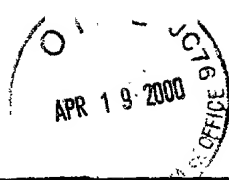
1           8. The process of claim 1, further comprising adding an earth alkali metal salt or  
2 an earth metal salt, or a transition metal salt to the separated fermentation liquor.

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## Abstract of the Disclosure

In a process for preparing mevinolin by fermentation of a biomass in a fermentation liquor, which includes dissolving mevinolin from the biomass into the fermentation liquor, and separating the biomass from the fermentation liquor to obtain a separated fermentation liquor, separating the mevinolin from the separated fermentation liquor, and recovering the end product, **the improvement which comprises** carrying out the dissolving at a pH between about 7.5 and about 10, and the separating of the mevinolin is carried out at a pH between about 4.5 and about 1.

005749-043900



DECLARATION WITH POWER FOR U.S. PATENT APPLICATION

ATTORNEY'S DOCKET NO. 100-

I, the undersigned inventor hereby declare that my residence, post office address, and my citizenship are correct as stated below following my signature; that to the best of my knowledge I am the first, original and joint inventor of the invention described and claimed in the application for United States Letters Patent, having the title **PROCESS FOR THE ISOLATION AND PURIFICATION OF MEVINOLIN**, the description and claims of which were filed in the United States Patent and Trademark Office simultaneously herewith; and I confirm that I reviewed and understand the contents of that filed specification and claims and recognize my obligation pursuant to 37 C.F.R. 1.56 to disclose all information that is material to the examination and prosecution of this patent application.

The first corresponding application anywhere was filed in Hungary, under No. P9203458, on November 4, 1993, the priority of which is hereby claimed.

This continuation in part application partially claims the priority of the following U.S. (international) parent application No. PCT/HU93/00051, filed on September 8, 1993. I recognize my obligation pursuant to 37 C.F.R. 1.56 to disclose all information that is material to the examination of this application, which information arose and came to my attention in the time period between the filing of the above-identified parent application and this application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made of information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

As a named inventor I grant power of attorney to the following attorneys to prosecute this application and to conduct any and all business in connection therewith in the United States Patent and Trademark Office, in this application, in any international application; and in the courts and before administrative agencies of the United States, and before the International Office:

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signed on behalf of the deceased János Bálint by the representative of his estate

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Residence: Debrecen, Hungary; Date: 4 May, 1994  
Mailing address: Fáy A. 21, H-4026 Debrecen, Hungary; Citizenship: Hungarian

Name: Ildikó Láng; Signature: Ildikó Láng

Residence: Budapest, Hungary; Date: 10 May, 1994  
Mailing address: Izabella 13, H-1077 Budapest, Hungary; Citizenship: Hungarian

Name: Imre Székely; Signature: \_\_\_\_\_

Residence: Debrecen, Hungary; Date: 31. May, 1994

Mailing address: Gyergyó 11, H-4028 Debrecen, Hungary; Citizenship: Hungarian

Name: Imre Moravcsik; Signature: \_\_\_\_\_

Residence: Budapest, Hungary; Date: 16. May, 1994

Mailing address: Mester 38, H-1095 Budapest, Hungary; Citizenship: Hungarian

Name: Vera Kovács; Signature: \_\_\_\_\_

Residence: Debrecen, Hungary; Date: 26 June, 1994

Mailing address: Cívis 3, H-4032 Debrecen, Hungary; Citizenship: Hungarian

Name: Szabolcs Mátyás; Signature: \_\_\_\_\_

Residence: Budapest, Hungary; Date: 18. May, 1994

Mailing address: Mária 34, H-1085 Budapest, Hungary; Citizenship: Hungarian

Name: Zsuzsanna Sztáray; Signature: \_\_\_\_\_

Residence: Debrecen, Hungary; Date: 7 May, 1994

Mailing address: Sumen 28, H-4024 Debrecen, Hungary; Citizenship: Hungarian

Name: László Eszenyi; Signature: \_\_\_\_\_

Residence: Debrecen, Hungary; Date: 4. May, 1994

Mailing address: Bekecs 14, H-4030 Debrecen, Hungary; Citizenship: Hungarian